

Effect of inhaled substance P and neurokinin A on the airways of normal and asthmatic subjects

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ABSTRACT The neuropeptides substance P and neurokinin A are present in sensory airway nerves. Their effect on airway calibre was compared in six healthy non-smoking subjects and six asthmatic subjects. On separate days increasing concentrations (from 10^{-9} to 10^{-6} mol/ml) of each neuropeptide were administered by nebuliser and the airway response measured as change in specific airway conductance (sGaw). Substance P and neurokinin A caused no change in sGaw in the healthy subjects. Inhalation of substance P up to the highest concentration of 10^{-6} mol/ml caused no change in sGaw in the asthmatic subjects. Neurokinin A, however, caused bronchoconstriction with a mean fall in sGaw of 48% (SEM 12%) after 5×10^{-7} mol/ml. The onset of bronchoconstriction was rapid, but sGaw had returned to baseline values within one hour in all but one patient.

Several neuropeptides have been detected in the airway by immunological techniques.¹ These include substance P and neurokinin A, two members of the tachykinin peptide family. These structurally related peptides are potent smooth muscle constrictors and blood vessel dilators.²

Substance P has been localised within sensory airway nerves of animals and man: nerve fibres with substance P like immunoreactivity have been seen in the airway mucosa; around blood vessels, submucosal glands, and tracheobronchial ganglion cells; and within airway smooth muscle.^{3,4}

Neurokinin A is a recently discovered mammalian tachykinin, whose structure is contained, with substance P, in one of the two mammalian tachykinin precursors, β preprotachykinin.⁵ Neurokinin A co-exists with substance P within peripheral sensory nerves,⁶ and its presence within sensory airway nerves was reported recently.^{7,8}

A potential role for tachykinins in asthma has been suggested recently.^{9,10} From experiments in rodents it was hypothesised that mechanical and chemical stimulation of sensory airway nerve endings cause antidromic release of substance P, neurokinin A and calcitonin gene related peptide from axon collaterals,

leading to smooth muscle contraction, mucus secretion, vasodilatation, and protein extravasation (axon reflex hypothesis). Substance P, however, when inhaled has only a weak bronchoconstrictor effect.¹¹ In animals and in isolated human airways, neurokinin A is a more potent bronchoconstrictor than substance P.¹²⁻¹⁴ We decided to compare the effect of inhaling substance P and neurokinin A on airway calibre of normal and mildly asthmatic subjects.

Methods

PATIENTS

The study population consisted of six healthy, non-smoking volunteers and six patients with a history of mild asthma. The normal volunteers, two men and four women, aged 24-35 years, had normal lung function (mean FEV₁ 115% of predicted, mean sGaw 1.92 (0.04) s⁻¹ kPa⁻¹). The asthmatic patients, four men and two women, aged 18-28 years, had only minor symptoms during the weeks preceding the trial. Their clinical characteristics are given in table 1. Five of the six asthmatic subjects had positive skinprick test responses, five to house dust mite and two to grass pollen. Their FEV₁ was greater than 70% predicted (mean 80 (SEM 3)) and their PC₂₀ (provocative concentration causing a 20% fall in FEV₁) methacholine less than 8 mg/ml. PC₂₀ methacholine was determined by measuring the FEV₁ decrease after inhalation of doubling concentrations of methacholine, according to the method described by Cockcroft *et al.*¹⁵

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Table 1 Clinical data on the six asthmatic subjects

Patient No	Age (y)	Sex	Weight (kg)	Basal FEV ₁ (% predicted)	sGaw (s ⁻¹ kPa ⁻¹)	PC ₂₀ (methacholine, mg/ml)	Skinprick test response	Treatment
1	18	M	65	74	0.79	2.60	D pt, gp	S
2	22	F	57	84	2.04	2.20	D pt, gp	T + S
3	22	M	91	93	0.91	4.00	D pt, gp	S
4	27	F	56	78	1.86	5.50	Negative	T + S + A
5	28	M	58	76	1.55	4.50	D pt	S
6	19	M	60	74	1.02	6.00	D pt	S

D pt, *Dermatophagoides pteronyssinus*; gp, grass pollen; S, inhaled sympathomimetic; A, inhaled anticholinergic; T, oral theophylline.

All subjects gave informed consent. The study design was approved by the ethical committee of the University Hospital of Ghent.

MATERIALS AND SOLUTIONS

Substance P was obtained from UCB Bioproducts (Brussels, Belgium), neurokinin A from Peninsula (St Helens, England), and human serum albumin from Behringwerke AG (Marburg, Germany).

The neuropeptides were dissolved in saline containing 1% human serum albumin. Saline containing 1% human serum albumin served as a control solution (pH 8.0, temperature 4°C, osmolality 320 mmol/kg). The neuropeptides were diluted freshly each day and kept on ice.

BRONCHIAL CHALLENGE

Subjects underwent a bronchial challenge with substance P, neurokinin A and a control solution in a randomised order on three separate days. Solutions were nebulised with a Wiesbadener-Doppel inhalator (Wiesbadener Inhalatoren-Vertrieb, Wiesbaden, Germany) with an airflow of 6 l min⁻¹. Increasing concentrations of substance P or neurokinin A were inhaled (10⁻⁹, 5 × 10⁻⁹, 10⁻⁸, 5 × 10⁻⁸, 10⁻⁷, 5 × 10⁻⁷, and 10⁻⁶ mol/ml) at 15 minute intervals. Control solutions were administered in the same manner. The nebulised solutions were inhaled during two minutes of tidal breathing with the outlet of the nebuliser in the mouth and the nose closed by a clip. Under these conditions 0.2 ml of the solution leaves the nebuliser. The three challenges were performed at an interval of at least one week. All medications taken by the asthmatic patients (sympathomimetics and theophylline) were stopped 24 hours before each challenge. The challenge tests were started at 2 pm.

LUNG FUNCTION MEASUREMENTS

The FEV₁ was measured with a water sealed spirometer (Expirograph, Godart) as the highest of three consecutive measurements. Specific airway conductance (sGaw) was calculated from airway resistance and thoracic gas volume measured with a constant volume body plethysmograph (Jaeger, Würzburg,

Germany). Measurements were made with the subjects breathing quietly under BTPS conditions, inhaling warmed, humidified air from a separate rebreathing bag to minimise temperature and humidity artefacts.¹⁶ Each value represents the mean of eight consecutive measurements. sGaw was measured before the start of the challenge and 5 and 15 minutes after the start of inhalation for each concentration. When sGaw had decreased by more than 35% it was measured every 15 minutes until it had returned to normal values.

STATISTICAL ANALYSIS

The results are expressed as the percentage change in specific airway conductance (Δ% sGaw), and reported as the means with standard errors in parentheses. The data from the two groups of subjects, the three solutions, and the different concentrations were compared by three way analysis of variance. Paired data were compared by the Wilcoxon's matched pairs signed rank test. A p value of <0.05 was regarded as significant.

Results

Inhalation of substance P and neurokinin A by the healthy, non-smoking subjects did not cause a significant change in sGaw (fig 1). Inhalation of sub-

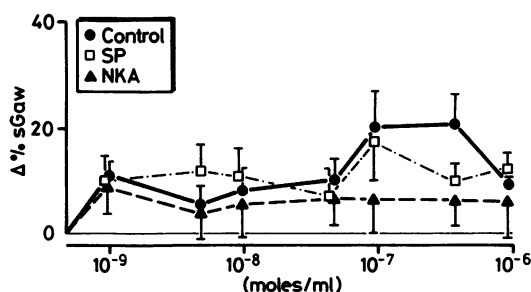


Fig 1 Percentage change (means with standard errors) in sGaw in six normal subjects five minutes after inhalation of increasing concentrations of substance P (SP) and neurokinin A (NKA, ▲).

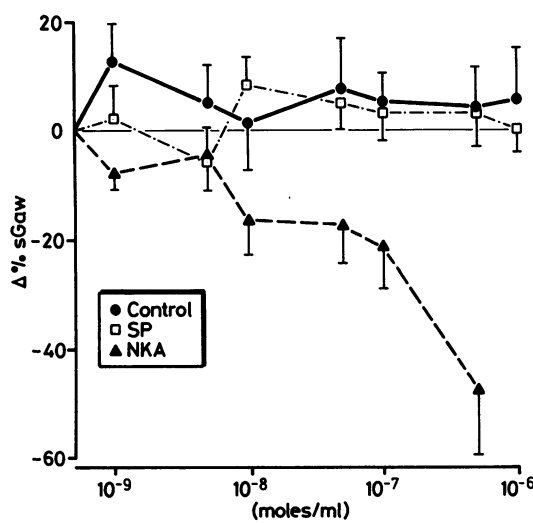


Fig 2 Percentage change (means with standard errors) in sGaw in six asthmatic subjects five minutes after inhalation of increasing concentrations of substance P (SP) and neurokinin A (NKA).

stance P in concentrations up to 10⁻⁶ mol/ml caused no significant change in sGaw in the asthmatic subjects. When the asthmatic subjects inhaled neurokinin A, however, there was a fall in sGaw (fig 2, table 2). The dose-response curve for the asthmatic subjects with neurokinin A was significantly different from the curves obtained with substance P and the control solution (ANOVA, p < 0.001). The decrease in sGaw after inhalation of neurokinin A differed significantly from change in sGaw after substance P and the control solution only at the concentration of 5 × 10⁻⁷ mol/ml (p < 0.05). At this concentration a fall in sGaw of more than 35% had occurred in five of the six asthmatic subjects (mean fall 48% (SEM 12%)). In the other patient (No 6) sGaw decreased by only 24% after inhalation of the highest concentration of 10⁻⁶ mol/ml.

Bronchoconstriction occurred rapidly in the five patients, with a > 35% fall in sGaw. The mean Δ% sGaw measured at 5 minutes was not statistically different from that measured at 15 minutes (table 2). sGaw had returned to baseline values within 30 minutes in three patients and within 45 minutes in a

further patient, but it remained low at one hour (Δ sGaw -62%) in one patient (No 5), whose initial Δ sGaw was -86%. His bronchoconstriction was rapidly alleviated by the administration of fenoterol. All patients with a fall in sGaw reported chest tightness. No change in heart rate or blood pressure and no cough occurred. Bronchial responsiveness to neurokinin A (PC₃₅) did not correlate significantly with bronchial reactivity to methacholine (PC₂₀) in the five patients with a reduction in sGaw greater than 35% (log₂ PC₃₅ neurokinin A mol/ml v log₂ PC₂₀ mg/ml: r = -0.48).

Discussion

In this study we have shown that the inhalation of nebulised neurokinin A caused bronchoconstriction in subjects with mild asthma. Substance P in concentrations up to 10⁻⁶ mol/ml had no effect. No change in specific airway conductance was seen after inhalation of substance P or neurokinin A by non-smoking healthy volunteers. Because of limitations in the solubility of the tachykinins, concentrations higher than 10⁻⁶ mol/ml were not given.

The absence of a bronchoconstrictor effect of substance P in our study is consistent with its rather weak effect on isolated human airway smooth muscle.^{17 18} Our finding that neurokinin A caused bronchoconstriction in asthmatic subjects correlates well with reports that it is a more potent bronchoconstrictor than substance P in animals^{12 14} and the recent finding that it is between 100 and 1000 fold more potent than substance P in contracting isolated human airways.^{19 20} Neurokinin A caused bronchoconstriction in the asthmatic patients, but had no effect in the normal volunteers. This suggests that bronchoconstriction was related to bronchial hyperresponsiveness. In the five subjects with a greater than 35% fall in sGaw no correlation was found between bronchial responsiveness to methacholine and neurokinin A. These studies need to be extended to a larger group of patients.

Substance P and neurokinin A are members of a family of neuropeptides, the tachykinins.² These are structurally related peptides, containing 10-12 amino acids, and sharing a common amidated C-terminal end (-Phe-X-Gly-Leu-Met-NH₂). Until recently substance P was the only tachykinin known to be present

Table 2 Mean percentage changes in sGaw (with standard errors in parentheses) after inhalation of neurokinin A in six asthmatic subjects

Concentration (mol/ml):	10 ⁻⁹	5 × 10 ⁻⁹	10 ⁻⁸	5 × 10 ⁻⁸	10 ⁻⁷	5 × 10 ⁻⁷
5 min	-8.2 (2.1)	-5.0 (5.4)	-17.0 (6.0)	-17.7 (6.9)	-21.8 (7.7)	-48.0 (11.8)
15 min	-7.5 (4.3)	-6.8 (5.2)	-6.3 (7.7)	-14.3 (8.7)	-12.2 (8.7)	-42.8 (11.0)

in mammalian nervous tissue. The other tachykinins were extracted from amphibians (for example, kassinin, physalaemin) and molluscs (for example, eledoisin).²¹ On the basis of the different rank of order of potencies displayed by various tachykinins when tested with a variety of bioassays, the existence of multiple substance P receptor subtypes has been proposed.²²⁻²³ On the SP-P (P for physalaemin) subtype, SP, physalaemin, eledoisin, and kassinin are more or less equipotent, while kassinin and eledoisin are much more active than substance P on the SP-E (E for eledoisin) receptor. Recently two new mammalian tachykinins, neurokinin A (= substance K) and neurokinin B (= neuromedin K) were discovered.^{5,24} Recent data have shown that these two new tachykinins are more potent on the SP-E than on the SP-P receptor subtype.²⁵ On the basis of animal studies¹²⁻¹⁴ and findings from isolated human airways^{19,20} a predominance of bronchial SP-E receptors has been postulated. Our results for human airways in vivo are compatible with this view, as neurokinin A was definitely more potent than substance P.

The mode of action of tachykinins in human airways is not known. In addition to bronchial smooth muscle contraction, substance P causes bronchial microvascular leakiness,²⁶ airway mucus secretion,²⁷ and histamine release from mast cells.²⁸ Experiments in rabbits and rats show that a considerable portion of the bronchoconstrictor effect of tachykinins arises from an interaction with cholinergic nerves, probably through release of acetylcholine from cholinergic nerve endings.^{14,29} Studies on isolated human bronchi suggested that substance P has a direct smooth muscle contracting effect, as substance P induced contraction is resistant to pretreatment with a histamine and muscarinic receptor antagonist.¹⁷ This observation cannot, however, be extended to human bronchi in vivo as nerve mediated responses are often lost during in vitro studies.^{30,31} Inhalation of capsaicin, a neurotoxin that damages sensory nerve fibres causing release of substance P, caused bronchoconstriction in man.³² The bronchoconstriction has a rapid onset (under 20 seconds) and short duration (under 60 seconds), and was found to be largely inhibited by the anticholinergic agent ipratropium bromide. It is, however, questionable whether the bronchoconstriction was due to release of substance P or another tachykinin: capsaicin has been shown to cause reflex bronchoconstriction in dogs³³ and the time course of capsaicin induced bronchoconstriction was very different from the time course that we observed after inhalation of neurokinin A by asthmatic patients.

In conclusion, our study shows that tachykinins are able to cause bronchoconstriction in asthmatic subjects. As in animal and isolated human airways, neu-

rokinin A is a more potent bronchoconstrictor than substance P, suggesting a predominance of SP-E receptor sites in human airways. Axon reflexes could therefore play a part in the pathogenesis of asthma. The natural stimuli that cause tachykinin release from sensory airway nerve endings are unknown at the present. Further studies are necessary to define the exact role and the mode of action of tachykinins in human airways.

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